AD NUMBER

AD839392

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; 1963. Other requests shall be referred to Department of the Army, Fort Detrick, Attn: Technical Release Branch/TID, Frederick, MD 21701.

AUTHORITY

Fort Detrick/SMUFD ltr dtd 14 Feb 1972

TRANSLATION NO. 1211

DATE: 1963

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

THE POSSIBILITY OF DETECTING BRUCELLA ANTIGENS BY MEANS OF FLUORESCENT ANTIBODIES IN GYNECOLOGY

K. Zak and Zd. Veznik

THE POSSIBILITY OF DETECTING BRUCELLA ANIGENS BY MEANS OF FLUORESCENT SNTIBODIES IN GYNECOLOGY

From the II Clinic for Obstetrics and Gynecology
(Governing body; Doz. Dr. M. Uher, C. Sc.)
and from the Research Institute for Veterinary Medecine,
Institution for Physiology and Pathology of Reproduction
in Brunn (CSSR)
(Governing body: MVDr. Zd. Veznik, C. Sc.)

1964 - [illegible]

Published in : Zentralblatt fur Gynakologie, 1963, vol. 30.

The problem og diagnosis in the bacterial and virus diseases in gynecology and obstetrics is always important. For that reason each new method, which can contribute to the solution of this problem deserves our attention.

The detection of antibodies or antigens with the help of the classical serological method is usually the indirect method, the evidence in-vitro; also the earlier methods, the fortune to pursue the antigens in animal bodies, or to detect the place where the antigens are forming was detected by the indirect method. Similarly also was the solution of making the anigens for example with atoxyl, iodine or radioactive isotopes P^{32}, J^{131} (Haurowitz 1932, Libby and Madison 1947, Warren and Dixon 1948).

The immediate proof of the union of antigens with antibodies can be carried out by optical organic material (Fluorochrome). Coons and Mitarb (1941) have gåven rise to immunofluoresence. With designated antigens the bacterial agents, the tissue cap or cut, or conversely the unknown serums are identifies. Likewise, this method can by the study of the

syn[illegible] of anti-bodies in the cells can be employed. [illegible] (1961) there have been published about 300 articles [illegible] this problem.

The antibody molecules are protein, which in the cells which are specialised for the process concerned are synthesised. These were separated in the cycle where they continue for one week under gradual diminution of their quantity. Its half life period is in the case of man about 13 days, in the case of rabbits 5 days (Coons 1960). By the same author is the special character of the structural drawing of a specific reaction sphere. On this basis, hitherto unknown, (Landsteiner, 1927, Coons 1960) the surfaces often react on the expressed molecule of antibodies with the molecule of the same antigen so that they perhaps always form the same configuration. Even this specificity was exploited.

Creech, Jones and Coons (1941) have proved that antigens in the phagocyte cells of the mouse can be made visible by means of fluoresence of designated anitbodies by specifics. For that reason fluoresence was originally chosen because in mammalian tissue the green fluorosing is missing and because it emits an intensive greenish-yellow light.

The quantitative action of fluoresence is according to Coons (1960) about 75%. Besides the duration of the wave which it emits (5200 A) corresponds to the maximum susceptibility of the retina. The original fluoresence utilized, a proportional unstable compound, has been replaced by fluores-

zeinisothiozanant the last time. It is more stable of a more compact material which can be added to the buffer solution of antibodies whereas Cherry (1960) as well as Coons (1960) maintain the proportion of 5 mg. of chemical preparation for 100 mg. of globulin protein.

The designated antibody solutions combine the practicability of morphology and immunology. Under favorable conditions a striking specificity of the reaction " antigen- antibody" exsists, and it can be identified with the help of a single bacterial cell in the mixed flora. Here to be sure, also, the quality which contains antibodies in the serum is active whose concentration and all for the conjugation of the necessary chemical reactions (Kaufman, Cherry 1961). Also the condition is important that the molecule of the antibody has reacted as soon as possible with the specific antigen, cannot be expressed through the salt solution which does not enter into the reaction. The reliability of the reaction is therefore also depeudent on which of the measures the control searched are accomplished..

As antigens, tissues can be used, whose cultures, contain viruses or bacteria, protozoa, mites or soluble antigens of different types. The colored preparationx are exsmened after necessary preliminary fixation and indeed after the infusion of buffered glycerol which has combined with antibodies, appears clearly fluorescent. The uncolored material remains almost invisible or it can produce autofluoresence of different degrees.

The direct coloring method is used, the inhibition (which is used for the control of specificity), the indirect coloring and the complimentary coloring. Each way of coloring requires a control of specificity in order that the reaction can be estimated accurately.

1. The non-infected tissue should not be colored with the marked serum;
2. neither the normal serum nor its conjugate should color the natigen;
3. the coloring should be prevented by the preliminary treatment of materials with no-colored antibodies.

Although a series of different pathological agents has been identified, the method can not be used as a standard test for unequivocal diagnosis. Up till now it was not possible, an arrangement with all easily used diagnostic tests were accomplished, and the FA-method itself is in evolution. To its benefit also belongs, the identification can then be undertaken, if the stimulaying agent of disease has already lost its vitality.

METHOD AND MATERIAL

For the identification of brucellosis infections in test animals by means of FA we have used in the hyperimune serum in 3 tests with an agglutination titer of 1:640+++. To infect the guinea pigs (12 animals) the Brucella-Stamm Bang NR 5587 was used, which isolated from a ground case and thanks to the kindness the preparation SVU was supplied to us in Brno (Civil analysis preparation). The fractionalting of the serum-

globulin was accomplished by means of ammonium sulfate establishing the total protein content in the serum by the buret method. The globulin was dissolved and dialyzes at a temperature of 0° to 5°C with 0.85% NaCl. After termination of the dialysis a solution of 1% was prepared and the Isothiozyanalfluorwszein was added in the corresponding ratio. For the purpose of eliminationg the color material the conjugate was agitated with the same range of Dowex 2-x4(Cloroform) 20 to 50 mesh; for the purpose of elimination of resin it was dialyzed against the buffered solution. Finally the conjugate was treated by repeated sorption on the homogenized liver producing a powder and frozen at -20°C.

After the first dialysis we have the conjugation and the enddialysis by means of the swift agglutination of the hyperimmune serum on the coverglass, which always produces a distinct positive result. After finishing the preparation we have controlled the agglutination titer of all 3 control serums whereby the following result:

I-1: 80+++
II-1: 40+++ (the conjugate was not used further)
III-1: 320+++

In the course of the experinent 12 guinea pigs were used: 5 of the animals were preganant.

The group of preganant and non-pregnant guinea pigs were infected the following ways:

a) intramuscular and during a period of 10 days.

b) intraperitoneal

The infection was applied to the cleansed culture. For the purpose of estimating the standard content of infected embryoes, the suspension of McFarlans nephelometrischen standard Nr4 was diluted; each time 0.5 ml. were dispensed. After two deliveries of the suspension of brucella the animals were dead within 72 hours; none have dies spontaneously, 2 animals aborted within 24 to 30 hours after peritoneal application.

A smear from the peritoneal puncture was obtained, further impression preparations or abrasions of the peritoneum, liver, lymphatic nodules and endometrium were obtained. In the case of the pregnant animals aslo preparations from the placenta or from the rest of the chorial elememeats. Further preparations were made from abrasion material from the uterine cavity and fromthe aborted fetus.

Similar preparations were made from the material of the control animals.

The inhibition test according to Goldmann was used for identification of specificity. The fluoresence was estimated at + up to ++++ (Cherry 1960).

RESULTS

In the tissue and smears of the infected animals the specific fluoresence has been demonstrated. The negative and inhibited control preparations show minimal not specific fluoresence.

DISCUSSION

The FA method is in the case of diagnosis of the sex tract hence also of brucellosis of great importance. The dependent principle in the rapidity of all supposition can be that it can be employed easily. Also the brucella were dedicated throurgh attention. Cherry (1960) mentioned 81 strains, including B-Abortus suis and mellitensis which were investigated by means of the method of designated globulin. These strains- from virulent to avirulent- were colored with the FAmethod. No significant differences were observed in regard to color reaction; the strains were killed by phenol, or cooked bacteria or the former after incubation of the culture under increased tension of CO_2 was produced. It was shown that the FA was not only homologous but also both strains stained. Fluoreseent brucella with the antigens were fixed on the smear which contained less than 250 cells and indeed also then, when they contained a series of contaminants. The indirect test was used by Cherry (1960) for establishment of brucella antibodies in the serum of animals and humans. Biegeleisen, Moody, Marcus, Flynt (1961) designated serum globulin for detection of antiserum from B suis, colored 58 cultures of B Abortus suis and mellitensis. Janny , Herman (1962) stained with designated antibodies B-Abortus and in the peritoenal exudate the same antigen in the case of the guineq pigs. Moulton abd Mayer (1960) have employed the technique of FA for identification in necrotic liver cells

which with B-suis infected guinea pigs.

The test copy proportion confirmed that this technique is most appropriate for the detection of brucella antigens in animal tissue. The tests are reliable assuming that in the suspension 2 X 10^2 micro-organisms per 1 ml occur, while in the agglutination tests 2 X 10^8 per ml. are necessary. Bergeleisen and Mitarb (1962) have used this method to isolate the brucella from airtests and are of the opinion that this method can be used in infected or colored tissues for <u>depis-tagezwecke</u>(?). Kramar (1962) has reported about his experiments with FA in the identification of toxoplasmosis. In Italy and other countries this method was used as <u>depistages-mittel</u>(?) for the diagnosis of gonorrhea.

The perspective use of the FA method also in the case of other infections of the sex tract have certainly been possible.

CONCLUSIONS

The method of fluorescence of antigens was applied for detection of brucella in different tissues of pregnant and non-pregnant guinea pigs. The brucella antigen was detected intra and extra-cellular in the tissues investigated. The inhibition test and investigation of non-infectious material have shown the specificity of the method used.

Fig. 1. A centrifugated peritoneal puncture. Leucocytes with the phagocytal antigen, with specific fluoresence.

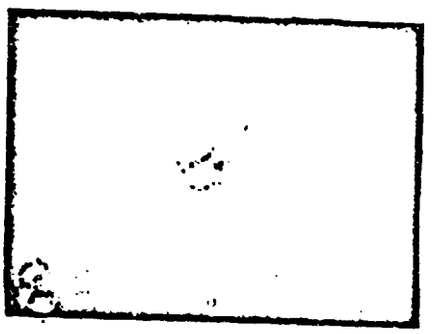

Fig. 2. In the group of special luminous peritoneal cells 2 markedly fluorescent leucocytes are apparent

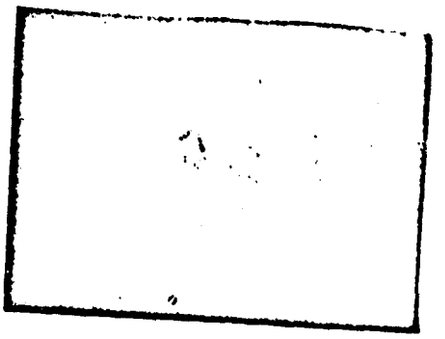

Fig. 3, Monocyte with the pahagocytic antigen in plasma, the nucleus does not fluores.

Fig. 4. A group of of endometr cells with the phagocyte, characteristically luminous antigen.

Fig. 5. The cells of trophoblast from thecurretage material after brucelhosis abordtion experiment; specific fluoresence.